**UNITED STATES DEPARTMENT OF COMMERCE**
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ID

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | | ATTORNEY DOCKET NO. |
|---|---|---|---|---|
| 09/388,899 | 09/02/99 | HOUWEN | B | 10690/T/B/A |

HM12/1120

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| EXAMINER | |
|---|---|
| GABEL, G | |
| **ART UNIT** | **PAPER NUMBER** |
| 1641 | 5 |

**DATE MAILED:** 11/20/00

**Please find below and/or attached an Office communication concerning this application or proceeding.**

**Commissioner of Patents and Trademarks**

| Offic Action Summary | Application N . | Applicant(s) | |
|---|---|---|---|
| | 09/388,899 | HOUWEN ET AL. | |
| | Examiner | Art Unit | |
| | Gailene R. Gabel | 1641 | |

*-- The MAILING DATE of this communication appears n the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

**Status**

1)☒ Responsive to communication(s) filed on *02 September 1999* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-11* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-11* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claims _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved.

12)☐ The oath or declaration is objected to by the Examiner.

**Priority under 35 U.S.C. § 119**

13)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

a)☐ All b)☐ Some * c)☐ None of the CERTIFIED copies of the priority documents have been:

1.☐ received.

2.☐ received in Application No. (Series Code / Serial Number) _____ .

3.☐ received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. & 119(e).

**Attachment(s)**

14) ☒ Notice of References Cited (PTO-892)
15) ☒ Notice of Draftsperson's Patent Drawing Review (PTO-948)
16) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4* .
17) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
18) ☐ Notice of Informal Patent Application (PTO-152)
19) ☐ Other: .

## DETAILED ACTION

### *Oath/Declaration*

1. The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because the date of signing by all Applicants is missing.

### *Claim Rejections - 35 USC § 112*

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

2. Claims 1-11 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 1 in line 12 has improper antecedent basis problem in reciting "in order to stain leucocytic cells". Change to --in order to stain the leucocytic cells (or the leucocytes)-- for correct antecedent basis.

Claim 1, step 4 has improper antecedent basis problem in reciting "defining neutrophilic cells". Change to --defining the neutrophilic cells-- for correct antecedent basis.

Claims 2-11 have improper antecedent basis problem in reciting "A method according to claim". Change to --The method according to claim -- for correct antecedent basis.

Claim 3 has improper antecedent basis problem in reciting "a group of all leucocytic cells". Change to --a group of all the leucocytic cells (or all the leucocytes)-- for correct antecedent basis.

Claims 8 is indefinite for using parenthetical symbols because it is unclear whether the limitations within the parentheses are a part of the claimed invention.

In claim 8, change "allophicocyanin" to --allophycocyanin-- for correct spelling of the term.

Claim 10 is vague and indefinite in reciting "a sample collected from a mammal by apheresis" because it does not specifically define what the sample is but rather recites a mode of collection for the sample.

Claim 11 has improper antecedent basis problem in reciting "after erythrocytes are removed". Change to --after the erythrocytes are removed-- for correct antecedent basis.

### *Claim Rejections - 35 USC § 102*

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

3. Claims 1, 3-4, and 7-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Hubl et al. (Cytometry, 1997).

Hubl et al. teach a monoclonal antibody-based flow cytometric method of classifying and counting leucocytes by performing a five-part leucocyte differential using a three-color staining procedure. Specifically, Hubl et al. added anti-CD45 conjugated with fluorescein isothiocyanate (FITC), anti-CD 14 conjugated with phycoerythrin (PE) /CY5, and a cocktail of antibodies including anti-CD16 conjugated with PE into whole blood samples for analysis (see Abstract). After incubation, erythrocytes are lysed using Grub-lyse, Facs-Lysing solution, or Ortho-Mube Lysing reagent. The flow cytometers measured side angle light scatter (SALS), forward scatter (FALS), and three color fluorescence intensity measurements (see page 73, column 1). Hubl et al. specifically teach that leucocytes are separated and identified based on the CD-45 expression and that basophil and eosinophil population can be separated by gating cells into the light side scatter and measuring CD-45 fluorescence intensity. On the other hand, eosinophil and granulocytes are separated and identified based on CD-16 expression (see page 75, column 2).

4. Claims 1-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Bowen et al. (Laboratory Hematology, 1997).

Bowen et al. teach abnormal patterns of expression of CD16 and CD11b antigens by neutrophils in bone marrow of patients using flow cytometric monoclonal antibody-based, three color immunofluorescence technique which permits simultaneous characterization of different cell populations (see Abstract). In the study of Bowen et al., bone marrow was aspirated into blood collection tubes, stained using different monoclonal antibodies, then erythrocytes were lysed using Ortho Lyse. The monoclonal antibodies include anti-CD45 conjugated with Tri-Color, anti-CD16 conjugated with FITC, and anti-CD11b conjugated with PE. Five parameters which include SALS, FALS, Tri-color, FITC, and PE were measured using flow cytometry. In data analysis, Bowen teach that granulocytic cells can be defined on the basis of intensity of side scattered light (SALS) and fluorescence intensity by fluorescence labeled leucocyte specific anti-CD45 antibodies wherein each gate can be set to include developing and mature granulocyte populations and exclude agranular leucocyte populations (blasts, monocytes, and lymphocytes) which inherently have lower SALS (see page 294, column 1). Bowen also confirmed that peripheral blood neutrophil populations within varying maturation levels (promyelocytes, myelocytes, metamyelocytes, and band cells) are quantified within the CD11b and CD16 regions because both CD16 and CD 11b normally increase during the maturation of granulocytes from promyelocytic stage to segmented neutrophil stage (see page 294, column 2 and page 275, column 1). Bowen further observed that the manual percentage of band to segmented neutrophils correlated well with CD16 expression

suggesting that in the course of granulocyte maturation, CD11b expression appears earlier and prior to the expression of CD16; therefore, anti-CD16 antibodies are more useful in defining granulocytes in later maturation stages than CD11b (see page 296, column 2). In conclusion, Bowen teach that simultaneous quantitation of SALS and fluorescent labeled monoclonal antibody binding to CD45, CD16 , and CD11b define highly reproducible developmental maturation patterns of the granulocytic leucocyte population series in flow cytometry.

5. Claims 1-10 are rejected under 35 U.S.C. 102(b) as being anticipated by Loken et al. (EP 0317516).

Loken et al. disclose a method and kit for classifying and counting lineages and stages of hematopoietic cells including leucocytes. Specifically, Loken et al. disclose adding to a sample of bone marrow a first monoclonal antibody labeled with a first fluorochrome which specifically binds all leucocytes, in this case, anti-CD45 antibody. A second fluorochrome labeled monoclonal antibody specific to a subset of the leucocytes such as granulocytes is further added, in this case anti-CD16 and anti-CD11b are used for their ability to distinguish between granulocytic lineage and myeloid maturation stages (see columns 2 and 6). Specifically, Loken et al. disclose assessment of myeloid maturation in bone marrow cells by staining with several monoclonal antibodies including anti-CD16, anti-CD11b, and anti-CD15 in combination with anti-CD45 (see column 9). Loken et al. teach that fluorochrome labels should be selected to have a

similar excitation energy level but distinct emission spectra. In this case, Loken use FITC and PE which are conjugated directly or indirectly to antibodies in three-color flow cytometric analysis for measurement of their fluorescence intensity (see column 4). Loken et al. use flow cytometry to distinguish between cell lineages by cell size, granularity, and distribution of antibody binding to cells: FALS and SALS are used to separate cellular lineages based upon their optical and physical characteristics then SALS provides an approximation of cellular granularity which then provides a function of the presence (or absence) of structures such as nucleus and granules in the cells (see column 3 and 5). In conclusion, Loken disclose that by combining intensity of light scatter (FALS or SALS) and fluorescence intensity by different fluorochromes, various cell lineages and stages can be distinguished.

6. Claims 1, 3, and 7-11 are rejected under 35 U.S.C. 102(b) as being anticipated by Jackson et al. (US 5,776,709).

Jackson et al. disclose a method for using flow cytometry for identifying and enumerating cells representing subpopulations of leucocytes in blood or bone marrow samples wherein a three-parameter gate can be established based on combined analysis of SALS, FALS, and fluorescence intensity (see column 6, lines 31-44). Jackson et al. disclose incubating the sample with at least two fluorochrome labeled antibodies including a fluorochrome labeled primary antibody that recognizes all leucocytes such as anti-CD 45 and fluorochrome labeled secondary antibodies which

recognize leucocyte subpopulations such as anti-CD13 which is specific for granulocytic or myeloid cells. The fluorochromes for conjugation with antibodies include FITC, PE, and PerCP which are excited by light of the same wavelength and emit light at separately detectable wavelengths compatible with flow cytometry (see columns 7 and 8). In addition, Jackson et al. specifically disclose that an erythrocyte lysing reagent such as ammonium chloride and FACS Lysing Solution can be added to the sample for lysis or removal of erythrocytes prior to labeling of the leucocytes (see column 9, lines 4-13).

### *Claim Rejections - 35 USC § 103*

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

7. Claim 11 is rejected under 35 U.S.C. 103(a) as being unpatentable over Hubl et al. (Cytometry, 1997) or Bowen et al. (Laboratory Hematology, 1997) in view of McCarthy et al. (Journal of Immunological Methods, 1993).

Hubl et al. and Bowen et al. have been discussed supra. Hubl et al. and Bowen et al. fail to teach staining leucocytes after erythrocytes are removed from the hematological sample.

McCarthy et al. teach a flow cytometric procedure for the determination of surface antigens on leucocytes in whole blood including use of FITC labeled anti-CD16 and anti-CD11b antibodies. McCarthy et al. teach that Ficoll-Hypaque or dextran sedimentation are commonly used to purify and separate peripheral blood neutrophils from other cells such as erythrocytes prior to labeling the neutrophils for flow cytometric analysis (see page 155, column 2).

One of ordinary skill in the art at the time of the instant invention would have reasonable expectation of success in separating and purifying leucocytes such as neutrophils from other cellular components such as erythrocytes using Ficoll-Hypaque and dextran sedimentation such as taught by McCarthy prior to labeling of leucocytes for cytometric analysis such as taught by Hubl and Bowen because McCarthy specifically taught that such procedures of cellular separation or removal from other cellular populations are conventional and well-known in the art so that an issue of when

such a purification or separation procedure is introduced into a method of flow cytometric analysis, i.e. before or after binding of a label to desired cells, is an obvious design choice.

8. No claims are allowed.

***Remarks***

9. Prior art made of record are not relied upon but considered pertinent to the applicants' disclosure:

Hubl et al. (Hematopathology, 1996) teach the value of CD16 expression for left shift detection and acute phase response in neutrophil granulocyte maturation.

Macey et al. (Journal of Immunological Methods, 1997) teach different leucocyte preparation procedures and performed flow cytometric analysis of CD45 and CD 34 antigen expressing cells.

Van Vlasselaer (US 5,840,502) discloses methods of enriching specific cell types and populations by density gradient centrifugation.

Terstappen et al. (US 5,646,001) disclose methods of affinity binding separation of one or more selected subsets of biological entities from a mixed cell population.

Festin et al. (Journal of Immunological Methods, 1994) teach multicolor flow cytometric analysis of the CD45 antigens which provide improved lymphoid cell discrimination in bone marrow and tissue biopsies.

Hashimi et al. (The American Journal of Medicine, 1984) teach cytofluorometric detection of chronic myelocytic leukemia supervening in a Patient with Chronic Lymphocytic Leukemia.

10. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gailene R. Gabel whose telephone number is (703) 305-0807. The examiner can normally be reached on Monday to Thursday, 6:30 AM - 4:00 PM and alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Long Le can be reached on (703) 308-3399. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Christopher L. Chin

CHRISTOPHER L. CHIN
PRIMARY EXAMINER
GROUP ~~1800~~ 1641

grg
November 14, 2000